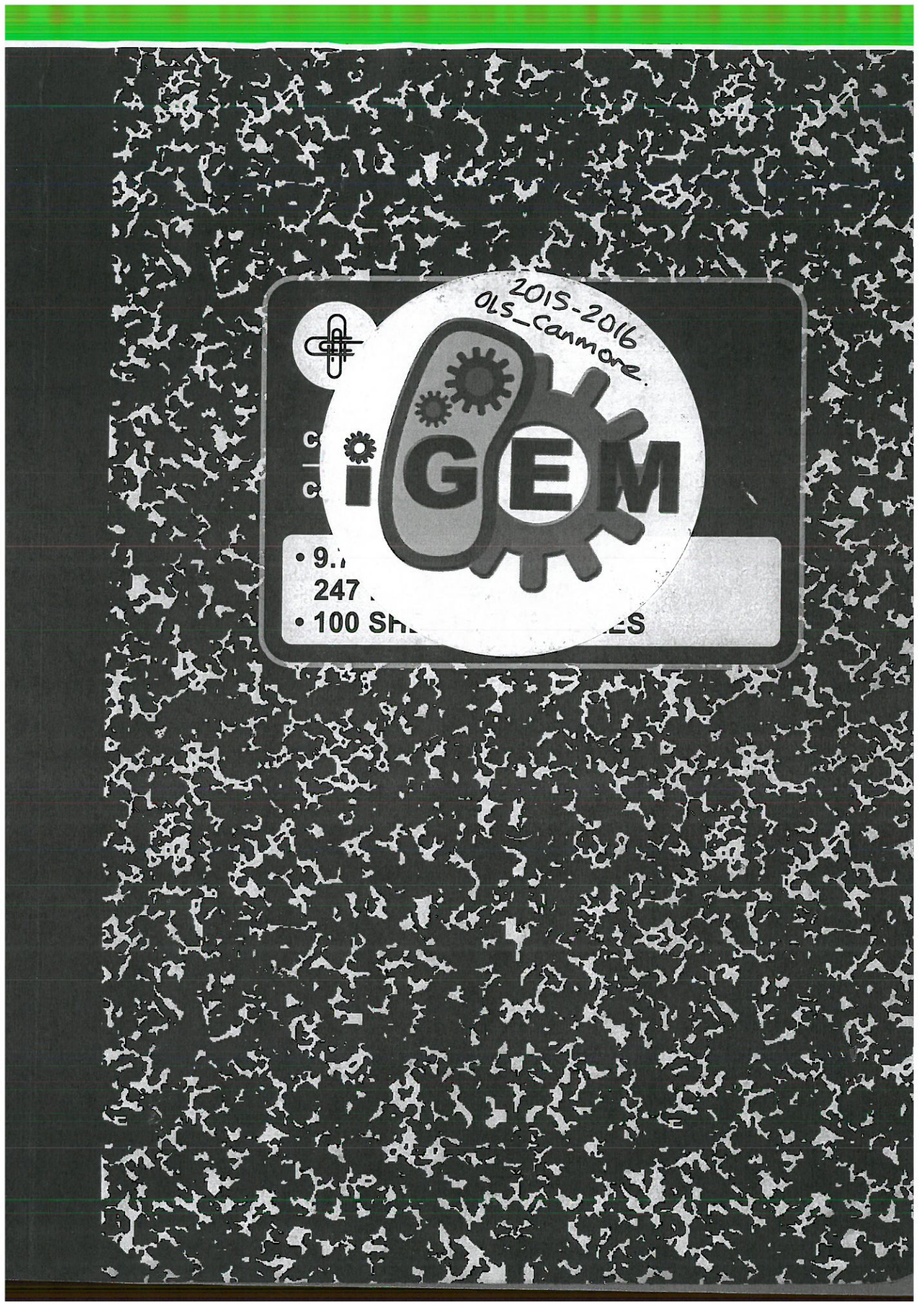

2015-2016
OLS_Canmore
iGEM

DATE: NOV. 20, 2015

TEAM MEMBERS: N/A

ADVISOR: J. PUHKUNEN

OBJECTIVE: Reconstitute synthesized KERA and KERUS plasmids received from Biobasic, prepare for storag

PROTOCOL FOLLOWED:

1) Centrifuged the DNA tubes on lowest speed setting as directed, to get any DNA attached to walls.

2) Re-suspended 10μg of DNA in 100μL of autoclaved ddH_2O. Final [DNA] = 100ng/μL. This was labele as ORIGINAL KERA/KERUS stock tubes.

3) In new, sterile eppendorf tube, made a 1:10 dilution of original stock (10μL original DNA stock into 90μL ddH_2O). Labelled as DILUTE stoc

Dec 3, 2015

Team members: T.D, N.S, O.N
Advisor: J.P
Objective: Grow NEB and TOP10 E.Coli culture from plates in preperation of making comp. cells

- Use an inoculating loop to pick a single colony and add the bacteria to 5ml of LB broth (in 10mL tube). Grow over night at 37°C with shaking. Make sure the tube is capped loosley to allow air to enter the tube. We repeted this step 4 times in
Tube 1 -> NEB C #1
Tube 2 -> NEB C #2
Tube 3 -> Top 10 C #1
Tube 4 -> Top 10 C #2

Dec 4th, 2015

Team members:
Advisor: J.P
Objective: Check Dec 3/15 coltures
Sub-coltures comp. Cell line in preparation to make comp cells.

Tube 1 (NEB-b C#1) -> Cloudy
Tube 2 (NEB-b C#2) -> Cloudy
Tube 3 (Top 10 C#1) -> Cloudy
Tube 4 (Top 10 C#2) -> Cloudy

- Sub-culture of 0.1mL (100µl) of NEB10 C#1, and NEB10 C#2 into 10mL of LB broth
Time = 8:20 am

Re-Planting:
onto LB-only agen Plantes 50µL of culture
1-NEBb C#1 or plate 1
2-NEBb C#2 or plate 2
3-Top 10 C#1 or Plate 3

5) Brought plates to room temp

6) Plates as follows:

a) 200μL herA dilute on LB-Amp
b) 200μL herA full on LB-Amp
c) 20μL herA full on LB-Amp
d) 200μL herUS dilute on LB-Amp
e) 200μL herUS Full on LB-Amp
f) 20μL " " on " "
g) 200μL RFP on LB-only
h) 200μL Ø on LB-Amp

Next day results:

Note- fan had been left on and many plates were very dry.

a) → no cells evident
b) → " "
c) → " "
} herA transformation all unsuccessful

d) → No cells evident
e) → " "
f) → " "
} herUS transformation unsuccessful

g) → Cells evident, Not red
h) → NO growth, as exspected
} comp cells viable but transform unsuccessful

Jan 12/2015

J. PUURUNEN

Sent Plasmid DNA to L. Oberding:

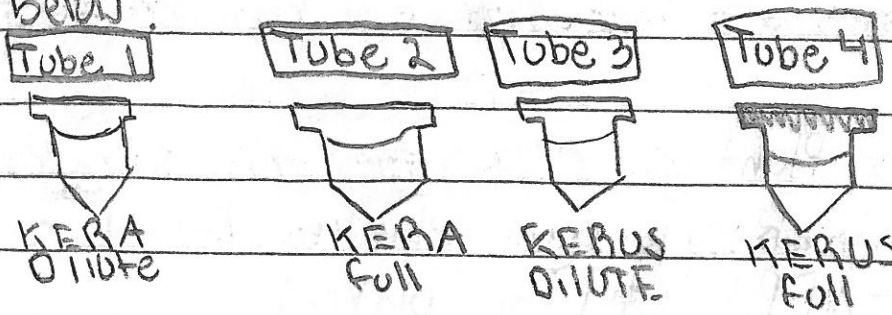
5µL KERA full (100ng/µL)
5µL KERUS full (100ng/µL)
5µL KERA dilute (1:10)
5µL KERUS dilute (1:10).

4-Top10 C#2 on Plate 4

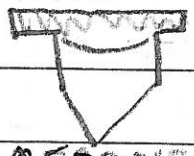
Dec 4, 2015 - 11:30am
LAB Members:
Advisor: J.P
objective create comp. cells for Immediat use.

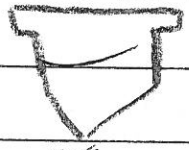
1. Removed sub-cultures from 8:30am, OD600 Spec results:
   A) NEB-b C#1 → OD600 = 0.521
   B) NEB-b C#2 → OD600 = 0.569

2. Transfered 500 µL bacterial culture from NEB C#1 tube to each 10f 6 1.5mL eppendorf tubes as show below:



Tube 5 Tube 6





REP 2015
plate 3
230

(-) Control

*each tube received 500µL of NEB C# 1 subculture

For each tube:
- Spin each tube at 7000 rpm for 2-3 min
- Discard supernatant
- resuspend in 50µL CaCl2, spin down again *50mM - we used 100
- resuspend in 100µL CaCl place on ice
- Transform immediately

Transformation:

1) *added 5µL of DNA to each of the tubes
*"0" negative control tube got NO DNA

2) Incubated tubes on ice for 30 min

3) incubated at 37°C for 5 min Then Placed immediately on ice for 5 minutes

4) Added 200µl LB medium and mixed gently by tapping the tube. Incubated at 37°C for 1hr and 40 min

5) Brought plates to room temp

6) Plates as follows:

a) 200μL herA dilute on LB-Amp
b) 200μL herA full on LB-Amp
c) 20μL herA full on LB-Amp
d) 200μL herUS dilute on LB-Amp
e) 200μL herUS full on LB-Amp
f) 20μL " " on " "
g) 200μL RFP on LB-only
h) 200μL Ø on LB-Amp

Next day results:

Note- fan had been left on and many plates were very dry.

a) → no cells evident
b) → " "
c) → " "
} herA transformation all unsuccessful

d) → No cells evident
e) → " "
f) → " "
} herUS transform unsuccessful

g) → Cells evident, Not red
h) → NO growth, as exspected
} comp cells viable but transform unsuccessful

Jan 12/2015

J. PUURUNEN

Sent Plasmid DNA to L. Oberding:

5μL KERA full (100ng/μL)
5μL KERUS full (100ng/μL)
5μL KERA dilute (1:10)
5μL KERUS dilute (1:10).

JAN 14/15 – JP.

OBJECTIVE: Begin Competent Cell Protocol.

① Filled 3 falcon tubes (15mL) w 5mL sterile LB broth.

② Inoculated broth w single colony from 3 places:

NEB (NEB) – JM109 – ~~Plate 1, C#1~~

" " Plate 1, C#2

" " Plate 2, C#1

③ Incubated @ 37°C w shaking for 20 hours.

Jan 15/16 – J.P. and Olivia N. – 8am.

① Placed 10mL sterile LB broth into a 15mL falcon tube : x2 tubes.

② Sub-cultured 0.1mL of overnight cultures

→ Plate 1, C#2 and

→ Plate 2, C#1

Placed in 37°C w shaking, approx 3h

RFP = Kit Plate 3, 2015 – P23.

Jan 15/16 – ____ am: JP + ___ + ___.

① Checked OD600 on spec:

Plate 1 C#2 = 0.305

Plate 2, C#1 = 0.329

* Chose to use _____ for comp cells.

② Transferred 500µL to a 1.5mL eppendorf x 6 tube

labels:
- A) KERA full
- B) KERA dilute
- C) KER US full
- D) KER US dilute.
- E) RFP (+ control)
- F) Ø (– control).

③ Spun 6 tubes at 7000rpm for 2 min.
Discarded supernatant.

④ Added 500µL $CaCl_2$ (50mM), re-suspended cells. Spun down again (2min, 7000rpm), discarded supernatant.

⑤ Resuspend cells in 100µL sterile $CaCl_2$ (50mM) + place immediately on ice.

JAN 15/16: TRANSFORMATION PROTOCOL:

① Obtain 100μL competent cell aliquots, thawed on ice.

② Add 4μL of following plasmids to each tube

A) KERA full
B) KERA dilute
C) KERUS full
D) KERUS dilute
E) RFP – (+) control.
F) Ø (no plasmid added – (–) control)

mix by gently flicking

③ Incubate on ice for 30 min

④ Incubate @ 42°C for 60s

⑤ Ice for 5 mins

⑥ Add 200μL LB medium

⑦ Incubate @ 37°C for 1hr. 5min

Plating:

Plate 100μL of each onto their respective plates, spread w spreader

JAN 16-19th > NO GROWTH ON ANY PLATES.

Date: Feb 5th 2016 - Making IPTG and Skim Milk Plates

① 100 mM IPTG

0.12g in 5mL Sterile distilled Water. Dissolve and filter Sterilize (using a 0.2 microliter filter). Store in aliquots at -20°C

② 0.2 g/mL Skimmed Milk (Scaled up from Lisa 500mL of agar instead of 100mL)

10g of dried Skimmed milk in 50mL of distilled Water. Dissolve and filter Sterilize. (Dissolve this stock in 500ml of agar)

③ LB Agar Plates

Weigh out and add the following into 1L glass bottle or Erlenmeyer flask:

- 5.0g Tryptone
- 2.5g Yeast Extract
- 5.0g NaCl
- 7.5g Agar

Add distilled Water to 500mL. The bottle must remain half empty to prevent the liquid from boiling over into the autoclave.

Mix by swirling. Don't expect all solid powder to dissolve, but make sure that none gets stuck to the sides of the bottle.

Cover the top with aluminum foil and secure it with autoclave tape. The cover must be loose enough to prevent pressure from building inside the bottle during autoclaving.

Autoclave for 20 min at a pressure of 15 PSI

Remove from autoclave and allow it to cool to about 55°C

Now add the ampicillin, IPTG, and skim milk stocks. Swirl to m

It is important that the antibiotics are not added while liquid is still hot. Make sure everything is added aseptically. Use a sterile pipette tip and flame the bottle.

Add the following to LB broth

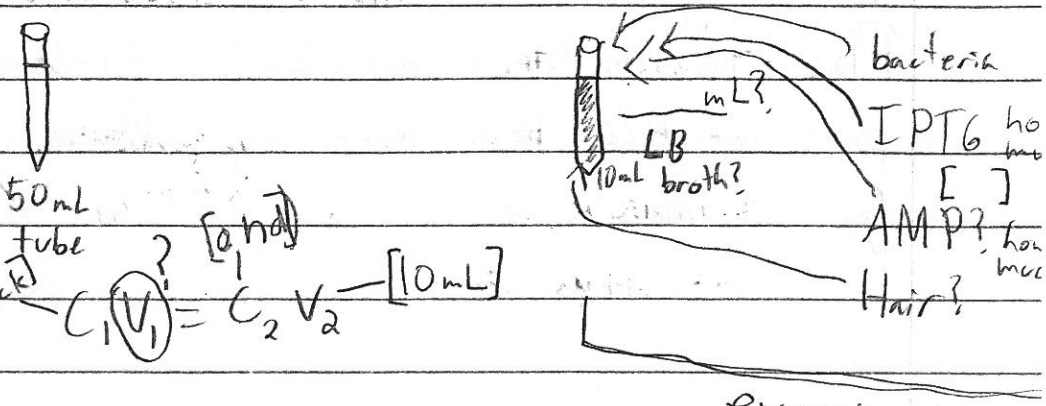
Stock:	Concentration:	Volume added:
Ampicillin	100 mg/mL	500 microliters
IPTG	100 mM	500 microliters
Skim Milk	0.2 g/mL	50 mL

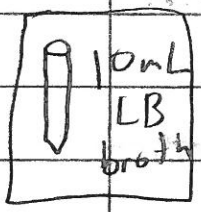
Pour or pipette about 20mL LB agar to each petri dish. This must be done asepectidly

Make Sure to flame the opening of the bottle and pour Slowly

Place lids on dishes and Cool for 30-60 min. Store LB plates at 4°C inside plastic bags. Plates will be good for a Month.

Feb 12 2016 Calculating amount of IPTG AMP, and hair, and concentration of IPTG





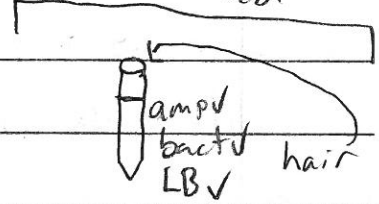
AMP

Stock Solution (freezer)

= [100mg/mL]

↳ Normally, we use 5μL in 5mL

So 10μL into 10mL of LB



IPTG = Stock Solution [100mM]

AMP = [100]

Ideal IPTG => 0.1 => 1mL

$[IPTG_{Stock}]$ ← $C_1 V_1 = C_2 V_2$ (10mL)

100mM

C_1 = 100mM, V_1 = ?, C_2 = 0.5mM, V_2 = 10mL

$(100\,mM)(V_1) = (0.5\,mM)(10\,mL)$

$(V_1) = \frac{(0.5\,mM)(10\,mL)}{(100\,mM)}$

$(V_1) = 0.05\,mL$

$= 50\,\mu L$

Feb. 23/16.

1) 5mL LB Broth + 5μL AMP stock solution:

- ↳ KERA C#1
- ↳ KERA C#2
- ↳ KERUS C#1
- ↳ KERUS C#2

} no growth for any tube.

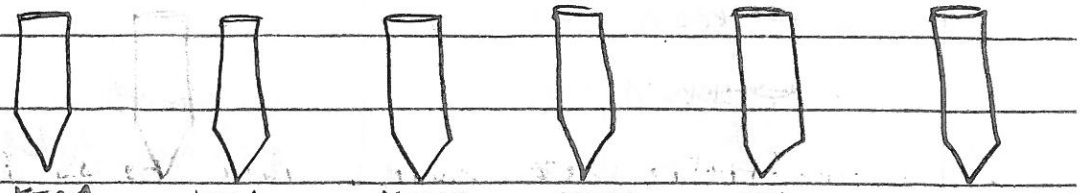
2) 3 plates - iGEM ethanol stab. JM109

- ↳ plate 1
- ↳ plate 2
- ↳ plate 3

} all grew single colonies well.

Feb. 24-26, 2016

OBJECTIVES: Prepare competent cells and transform them with the following plasmids:



Feb 24th:

① Pluck single colonies of JM109 (Feb 22 plates) and inoculate 3 separate tubes containing 5mL LB-only sterile broth.

② Grow overnight w shaking @ 37°C.

↳ note: had to ABORT this protocol due to a gas leak in the lab. Will Re-attempt soon.

MARCH 4/16 - F.P.

Objective:

MARCH 4th: J.P,

OBJECTIVE: Troubleshoot why transformed KERA + KERUS E. coli are not growing in LB-Amp culture.

ATTEMPT #1:

SUCCESS

- Re-plating KERA Plate 1 and KERUS Plate #2 by swiping 1/4 of old plate in inoculation loop + Re-streaking new LB-Amp. plates.
- Incubating @ 37°C overnight.

NO SUCCESS

ATTEMPT #2:

- Try culturing in LB broth with 50% Ampicillin concentration.
- Culture a single colony into 5mL LB-broth + 2.5μL 100mg/mL Amp stock solution.
- Incubate overnight @ 37°C.

- MARCH 10-11, 2016.
- OBJECTIVE: Make Fresh Competent Cells and Re-Transform KERA and KERUS.
- TEAM MEMBERS: D. Nowlan, J. Puurunen

① Plucked Single colonies of JM109 (from Biobasic) and grew in 5mL LB-only broth overnight @ shaking @ 37°C.

② 8:05am - Sub-cultured 100µL of overnight cells into 10mL LB-only broth. Grow @ 37°C @ shaking for 3 h. (x2 cultures)

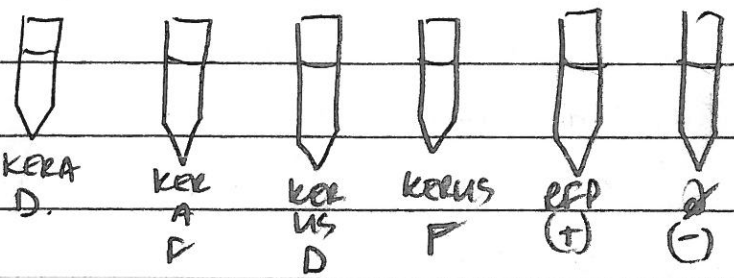
③ Check OD600 on each:

C#1 OD600 = 0.729.

C#2 OD600 = 0.709. used this

④ Transfer 500µL of C#2 subculture into each of 6 1.5mL eppendorf tubes, labelled as:

TUBE 4



(5) Spin each tube @ 7000 RPM for 3 min.

(6) Discard supernatant, don't disturb pellet.

(7) Resuspend pellet in 500µL sterile $CaCl_2$ (50mM).

(8) Spin down again 2-3min @ 7000RPM, discard supernatant.

(9) Resuspend all pellet in 100µL sterile $CaCl_2$, place on ice. → (Ready to transform!)

(10) using a sterile pipette tip and add 4µL ligation mix to competent cells. Mix gently by flicking the tube. → (Place on ice 30min.)

(11) Incubated @ 37°C for 5 min.

(12) Immediately on ice for 5min.

(13) Add 200µL LB-medium + mix gently.

(14) Incubate @ 37°C for 1.5 hours.

(15) Plated 75μL of each transformation onto appropriate plate
(AMP – for (–) controls, KERA / KERUS
(CHLOR – for (+) RFP control)

(16) Grow overnight @ 37°C.

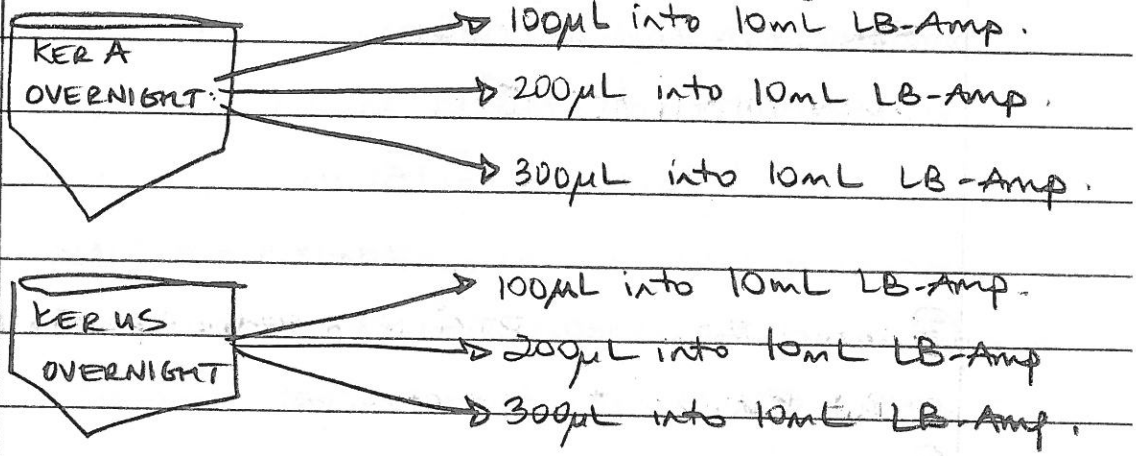
RESULTS:

KERA DILUTE – several colonies – poorly plated
KERA FULL – no growth.
KERUS DILUTE – several colonies – (20).
KERUS FULL – several colonies (≥40)
(+) RFP – no growth. (Chlor plate?)
(–) Ø – no growth.

MAR 18/16 – 7:50am – J. PUURUNEN.

OBJECTIVE: Sub-Culture KERA/KERUS cells, in prep for a quantitative hair digestion Test.

① SUB-CULTURE SET-UP: From 7:50–11:50am, sub-cultured the following from overnight cultures.



@ 11:50am: OD600 Measurements:

- KERA 100µL sub culture OD600 = 0.961
- KERA 200µL sub culture OD600 = 0.977.
- KERA 300µL sub culture OD600 = 1,032.
- KERUS 100µL sub-culture OD600 = 0,825
- KERUS 200µL sub-culture OD600 = 0,847.
- KERUS 300µL sub-culture OD600 = 0.907.
- KERA overnigh OD600 = not measured
- KERUS overnight OD600 = not measured.

② 10mL from each of the above cultures transferred into new falcon tubes

Mar 18/16 - 1:00pm - C. Nowlan

Calculation for volume of IPTG required for 10mL of LB broth:

IPTG: $C_1 V_1$ = LB-broth: $C_2 V_2$

$(100\,mM)(?) = (0.5\,mM)(10\,mL)$

$(?) = \frac{(0.5\,mM)(10\,mL)}{(100\,mM)}$

$= 0.05\,mL$ or $50\,\mu L$

(3) Insert 50 µL of IPTG (from 100mM stock) into each 10mL culture.

(4) portion out clumps of hair with a mass of 0.08 g (0.075g - 0.08g). Tie each clump of hair into a knot (2-3 knots are required to keep hair together).

(5) Place one knot of hair into each 10mL culture.

① Ker A 100 µL	② Ker A 200 µL
③ Ker A 300 µL	④ Ker US 100 µL
⑤ Ker US 200 µL	⑥ Ker US 300 µL
⑦ Ker A overnight	⑧ Ker US overnight
⑨ LB-only	⑩ JM109 - no Ker plasmid

Use an innoculating loop (flame sterilized between each addition) to push hair bundle into culture.

(6) pipette 50 µL of the 100 mM stock solution

(6b) Photograph EACH tube - Before Image.

(7) Place in incubator at 37°C overnight.

March 19th 2016 - 8:45-9:30am - Alina Arvisais

Objective: Record results from qualitative hair digestion test.

Results: No hair was degraded in any of the cultures. Pictures were taken to compare with yesterday's but if there was any degradation it was very minimal.

(1) Returned cultures to incubator at 37°C to leave for two more days (until next monday morning).

April 22nd 2016 - 9:15 - 9:30 am Naoto Isayama

Objective: Make 500mL LB broth, aliquot in 10 50mL containers.

Results: Successfully made 500mL of LB brot aliquoted in 10 50mL containers.

Group: Josh L., Nicholas S.

① Weigh out and add the Following into a 1L glass bottle or Erlenmeyer flask:

- 5g Tryptone
- 2.5g Yeast Extract
- 5g NaCl
- ~~7.5g agar~~

② Add distilled water to 500mL. The bottle mu remain half empty to prevent the liquid from boiling over in the autoclave.

③ Mix by swirling. Don't expect all solid powd to dissolve, but make sure that none gets stuck to the sides.

(4) Cover with aluminium foil and secure it with autoclave tape. The cover must be loose enough to prevent pressure from building inside the bottle during autoclaving

(5) Autoclave for 20 min at a pressure of about 15 psi.

(6) Remove from autoclave and allow it to cool to about 55 °C.

Apnl. 22. 2016. 9:30 - 9:45 am, Acacia

Objective: make 500 mL LB agar for 20-25 LB agar plates.

Results: Successfully made 500mL of LB agar.

Group: Maria B. Acacia M. Jadon O

OBJECTIVE - Make 500mL.

Protocol:

1. Weigh and add:

5g tryptone

2.5g Yeast

5g NaCl

7.5g agar

into a 1L glass bottle/Erlenmeyer flask.

2 Add distilled water to 500mL.

Bottle must remain half empty to prever the liquid from boiling over in the autoclave.

3. Mix by swirling. Make sure none gets stuck to the sides of the bottle.

4. Cover the top with aluminum foil and secure it with autoclave tape Cover must be loose enough to prevent pressure from building inside the bottle during autoclaving.
5. Autoclave for 20 min at 15 psi
6. Remove from autoclave and allow to cool to ≈55°C.

APR. 22/16 - Sasa, Chasey, Blaise, Olivia, Lisa

OBJECTIVE: Miniprep KERUS plasmids.

1. Grew overnight KERUS cultures (A+B).
2. Followed the OMEGA ERNA MINIPREP KIT protocol,
   Except: Step 14 - We did NOT add Elution Buffe
   we did 100µL of sterile, deionized H_2O.
3. Stored 4 miniprepped tubes (2 from each colony)
   of plasmid DNA @ -20°C

Troubleshoot
- 30mL gel.
- pH THE = (7) ←
- try 2 of miniprep tubes
- try 75mV, not 90.

Next time, use 50mL of TAE, 0.5g agarose.

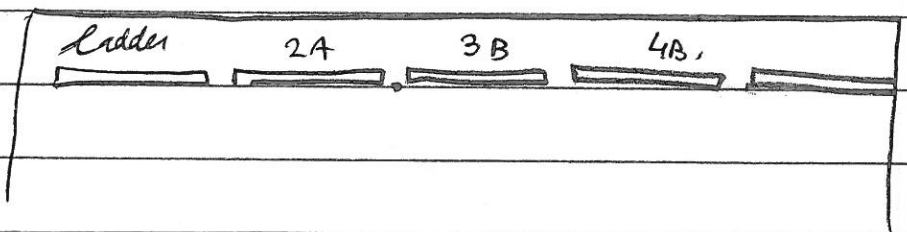
APR. 22/16 – Gel Electrophoresis

OBJECTIVE: Check plasmid DNA from minipreps today

1. Mixed 75mL of 1xTAE buffer with 0.75g of agarose powder in a 250mL Erlenmeyer.
2. Place flask in microwave, microwave on high for 1 minute, pausing every 10-15s to give flask a few swirls. Repeat until agarose dissolves
3. Allow agarose to cool until warm but not hot to touch.
4. Add 7.5μL RedSafe nucleic acid stain to agarose when cool.
5. Pour into electrophoresis mold (add combs!) and allow to set completely.

WELL LOADING:

1A – 10μL DNA + 2μL loading dye
2A 8μL DNA + 2μL loading dye
3B 6μL DNA + 2μl loading dye
4B 6μL DNA + 2μl loading dye.



PROTOCOL FOR MAKING SKIM MILK PLATES:

PROTOCOL #1:

1. Dissolved 5g skim milk powder into 125mL dH_2O Stir well w magnetic mixer.
2. Make 125mL LB-agar according to guidebook protocol.
3. First - autoclave the 125mL LB agar for 25min.
4. While LB agar cools, autoclave milk → heated only about 10 min (reached just below green zone)
5. When cool, add 2.5μL Amp to the LB-agar
6. Add skim milk to LB-Amp agar, swirl.
7. Pour plates.

↳ Plates looked very good - opaque-white, no solids.

* Allowed one "Sterile" plate to sit over the weekend @ room temp

PROTOCOL #2 - skim milk plates.

1. Dissolve 5g skim milk powder into the rest of the ingredients from LB-agar protocol - total volume = 250mL.
2. Autoclave entire mixture 25 min.
3. When cooled, add 2.5μL Amp.
4. Pour plates, let solidify.

↳ Autoclaved milk/agar mix had many solid, white chunks floating in it - (appeared to be curdled milk). As plates solidified, these chunks settled to BOTTOM.

→ possibly agar mixture is too acidic?

→ possible the autoclaving is cooking milk.

May 6th 2016 — Gel Electrophoresis

Josh, Alina, Naoto, Acacia

① Prepared gel according to geekstarter protocol (but scaled down to 40 mL.

② Added 2μL loading buffer/dye to 8μL DNA of each:

[1] DNA Ladder

[2] Ker us 1A

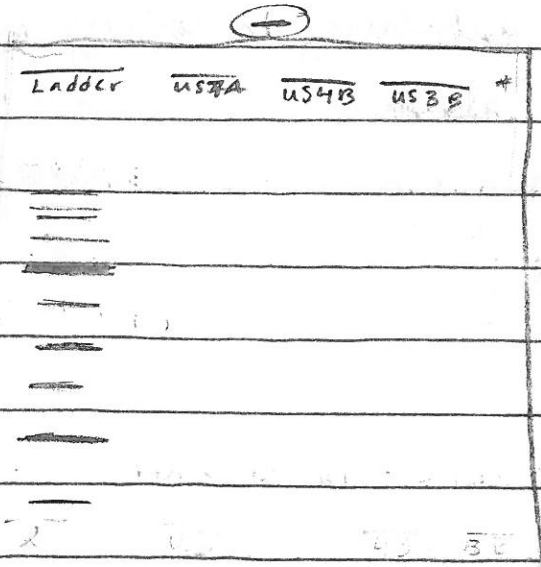
[3] Ker US 4B

[4] Ker us 3B

} from april 22rd workshop

There were some issues loading the gel, with some of the DNA bubbling out of the wells, which is why the third KerUS sample was used (opposed to the original plan of just two.

④ Results are as follows: Ladder showed nicely, but no bands could be seen in any of the other wells.



* there were more empty wells in the gell on the right side

All pictures will be uploaded to the lab results folder on google drive (linked in the classroom)

May 10th 2016 — Antibiotic Stocks (amp) and Overnight Cultures

Talia D., Alina A, and Mrs. P.

Objectives: 1) prepare new ampicillin antibiotic stocks 2) and prepare overnight cultures (2 each of KerA, KerUS, and JM109).

Objective 1

① New ampicillin stocks were prepared according to the geekstarter guidebook protocol.

Results: due to some spilling from the filter only 9 1.0mL stock solutions were created (compared to the usual 10)

Objective 2

① Prepared overnight cultures according to the geekstarter guide book protocol. However, the amount of LB and antibiotic solution was doubled to produce 10 mL cultures, instead of 5mL cultures.

② Six cultures were prepared:

1 JM109 – C7 (from Feb 23rd plate #2)
2 JM109-C8 (from Feb. 23rd plate #2)
3 Ker A – C2 (from April 11th)
4 Ker A – C3 (from April 11th)
5 Ker US – C2 (from march 18th)
6 Ker US - C1 (from march 18th)

Results: Both KerUS cultures looked cloudy and JM109-C7 looke cloudy, but everything else was clear.
OD600 measurements are recorded in the next entry.

May 11th 2016 — Beginning the Hair Degradation Assay

Alima A, Talia D, Maria B, Acacia M

Objective: begin the hair degradation assay for the biotreks research paper

① Measured the OD600 from all cultures from May 10

KerUS-C2 → 0.929 JM109-C8 → 0.016

KerUS-C1 → 0.567 Ker A-C2 → 0.001

JM109-C7 → 0.843 Ker A-C3 → 0.033

② Added 100 µL of 100 mM solution of IPTG.

③ Dried the hair with a hair dryer (at 64°C) and added 0.05g of hair into the KerUS C1+C2, and JM109 C7 cultures. The 3 remaining cultures were placed back in the incubator in the hopes of having KerA to re-plate.

④ photographed each falcon tube and placed in the incubator at 37°C with gentle shaking. More pictures will be taken tomorrow (May 12th) morning.

May 14th 2016 — Lethbridge Lab Workshop

Talia D., Josh L., Naoto I., Chasey K., Alina A., Luc A., David.

Objectives: mini-prep overnight KerUS cultures from Thurs and run a gel with mini-prepped DNA.

① Followed mini-prep protocol from the spring 2016 geekstarter Synthetic Biology workshop. (see protocol booklet)

Prepared 4 mini-preps:

① KerUS 1

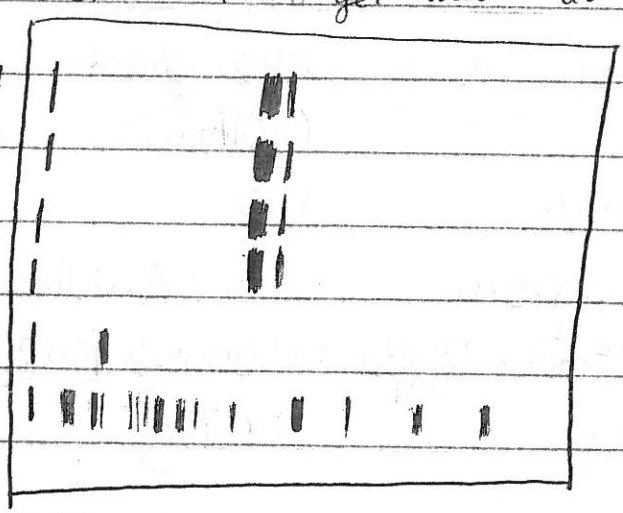
② KerUS 2

③ KerUS 3

④ KerUS 4

② Followed the gel electrophoresis from the spring 2016 geekstarter synthetic Biology workshop.

Layout of the gel was as follows:



* KerUS 3 was put into gel twice, because the first time it bubbled from the well.

1 2 3 4 ← did twice one after the other

May 11th 2016 — Beginning the Hair Degradation Assay

Alina A, Talia D, Maria B, Acacia M

Objective: begin the hair degradation assay for the biotreks research paper

① Measured the OD600 from all cultures from May

KerUS-C2 → 0.929 JM109-C8 → 0.016

KerUS-C1 → 0.567 Ker A-C2 → 0.001

JM109-C7 → 0.843 Ker A-C3 → 0.033

② Added 100 µL of 100 mM solution of IPTG.

③ Dried the hair with a hair dryer (at 64°C) and added 0.05g of hair into the KerUS C1+C2, and JM109 C7 cultures. The 3 remaining cultures were placed back the incubator in the hopes of having KerA to re-plate.

④ photographed each falcon tube and placed in the incubator at 37°C with gentle shaking. More pictures will be taken tomorrow (May 12th) morning

May 14th 2016 — Lethbridge Lab Workshop

Talia D., Josh L, Naoto I., Chasey K., Alina A, Luc A., David

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Prepared 4 mini-preps:

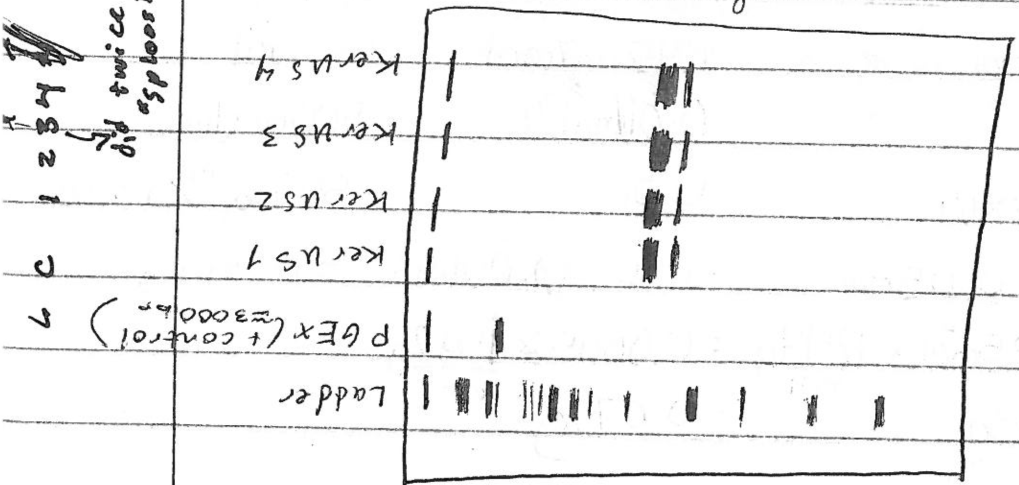
① KerUS 1

② KerUS 2

③ KerUS 3

④ KerUS 4

② Followed the gel electrophoresis from the spring 2016 geekstarter synthetic Biology workshop.
Layout of the gel was as follows:



* KerUS 3 was put into gel twice, because the first time it bubbled from the well.

May 15th 2016 - Lethbridge Lab workshop

Freya M, Maria B, Acacia M, Nick S, Naoto I, Olivia N, (Dora = Mentor)

Objectives: Lysing KERUS overnight cells for use in SDS page gel in afternoon

when diluting we because killed DH5α put amp in all of them.

① O/N KERUS / KERA + DH5α ctrl

② dilute 1/100 and add IPTG at 1mM (or add H_2O,

5mL LB + 5μL AMP + 50μl IPTG or H_2O

KerUS C4 - IPTG	KerUS C4 - H_2O
KerUS C3 - IPTG	KerUS C3 - H_2O
DH5α 1 - IPTG	DH5α 2 - IPTG
DH5α 1 - H_2O	DH5α 2 - H_2O

} one IPTG one H_2O (only 1 culture accidentally ~~exploded~~ extra tube(s))

9:46

③ Incubate at 37° for 3-6 hours

↳ started @ 9:46 → start lysing @ 12:46

④ ~~Spin at 7000 RPM for 8 min~~, add lysis buffer

Lysing the DH5α overnight culture not diluted because used AMP in dilution

Lysis Buffer: → Dithiothrietol

Tris:	DTT:	Lysosyme:
121.4g/mol	154.26g/mol	1mg/ml
0.5L	0.001mol/L	500ml × 1mg/ml = 500mg / 1000
0.05mol/L	0.5L	
0.5L × 0.05mol/L = 0.025mol × 121.14g/mol = 3.03g	0.5L × 0.001mol/L = 0.0005mol × 154.2g/mol = 0.077g	= 0.500g

KERUS C4 IPTG
KERUS C4 H_2O
~~KERUS C3/C5 IPTG~~
KERUS C3/C5 H_2O
DH5α overnight culture
Spun 1.5 mL of each
2× ↳ make bigger pellet

(5) shake for 30 mins in fridge
freeze/thaw liquid nitrogen & 40°C - 42°C water bath x2

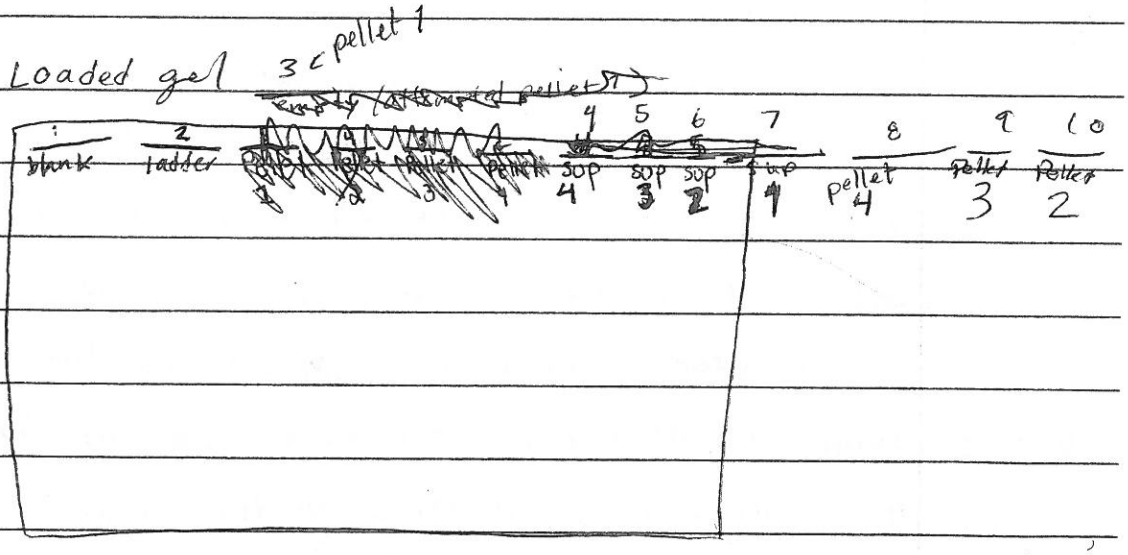
(6) centrifuge at 7000 RPM for 2 min x 2
added 60µl buffer, spin @ 7000 RPM for 2 min

8 tubes → 3 KERUS C4 IPTG → supernatant (sup) / pellet
2 KERUS C3/C5 IPTG → sup / pellet
1 KERUS C4 H_2O → sup / pellet
4 KERUS C3/C5 H_2O → sup / pellet

Added 5mL 8M Urea to pellets

(7) Added 3mL stain to each sample

(8) Loaded gel



⑨ Leftover supernatant was plated onto a skim milk plate (10 µL of each sample). Was left at room temperature for the 4 hour bus ride, then incubated at 37°C.

* Return PH w HCl? to neutral?

10 µL

Monday May 16th 2016 — Finishing Hair Degradation Assa

Talia D., Alina A.

Purpose: collect results from the assay started on wednesday

① Pictures of all cultures were taken before any further steps.

② Tubes were centrifuged at 3,000 RPM for 6 minute This was done to cause the hair to pellet. which made it easier to pour out the broth.

③ Hair was rinsed in 10% bleach. Bleach was poured out and clump of hair was placed in individual Petri dishes (using an innoculating loop)

④ Hair in Petri dishes was placed in the incubator at 37°C overnight to dry.

Results / Notes:

For future information:

① Not centrifuge the hair because it didn't do much and then it would be possible to measure the OD600 at the end b/c cells would not be at the bottom.

② Would also rinse hair with water after bleach so that when everything dries there is no residue left.

③ hair removal was difficult so perhaps not be so concerned to remove as much bleach (or in the future, water).

An update on the skim milk plates:
No clearing could be seen at all, but some colonie could be seen growing, therefore the plates were left in the incubator for another day to hopefully see ~~the~~ some clearing tomorrow.
Cultures growing was also a bit weird because the on things that were plated were ~~the~~ from the supern from the lysis only, but because of the Amp on t plates, the cells would have to be anti-biotic resistant

* also, there were some black "specs" found in all 3 tubes
* some pink discoloration was seen in the two KerUS tubes

Tuesday May 17th 2016 — Weighing Hair + Skim Milk Plates

Talia D., Alina A.

Objective: measure the plates of dried hair and check on the skim milk plates

① Hair + plates were weighed and data was recorded

② Checked on skim milk plate and took pictures of that and the hair.

Results:

Skim Milk: We saw some clearing today around the KerUS C4-IPTG supernatant and a tiny bit of clearing was seen by the KerUS C8-H_2O. They were kept in the incubator overnight again.

Hair:

Culture	Mass of Plate	Mass of Hair+Plate	Mass of Hair (calculated)
KerUS C1	17.54g	17.58g	0.04g
KerUS C2	17.86g	17.90g	0.05g
JM109 C7	17.92g	17.97g	0.05g

Only one culture showed a change (decrease) in the mass of hair but that was likely due to loosing some hair along the way and not a strong indication of hair degradation.

Also was not mentioned yesterday, but the KerUS tubes were pink-ish (but started white like the JM109 tube).

Thursday May 19th 2016 – Culturing KerUS+JM109

Objectives: culture some KerUS+ JM109 with LB-brot to dilute + induce and plate on skim milk plates tomorrow.

Note:

everything done with a flame/ aseptically

(1) Added 5uL of Amp stock solution to 5mL of LB-broth.

(2) Plucked single colonies from one KerUS plate and one JM109 plate:

[1] May 6/16 - KerUS Mar 18th re-streak ~ C3

[2] Feb 23/16 - LB only - JM109 - Biolabs ~ C8

(3) Placed in the incubator at 37°C overnight.

Results (from Friday morning): Both cultures grew and are very cloudy. We won't measure OD600 as we're just going to dilute and grow them up again for 3-6 more hours.

Friday May 20th 2016 — Skim Milk Plate Assay

Chasey K., Nick S., Talia D., Alina A.

objectives: dilute and induce cultures of KerUS + JM109

50 µl
40 µl
5 µl

① Labelled 6 tubes and added 5mL LB to each:

1 KerUS C3 - IPTG
2 KerUS C3 - H_2O
3 KerUS C4 - IPTG
4 KerUS C4 - H_2O
5 JM109 C8 - H_2O
6 JM109 C8 - IPTG

10 µl

② Added 5µl of amp to all KerUS culture tubes, then 50µl IPTG to the three "IPTG" cultures, then 50µl H_2O to the "H_2O" cultures.

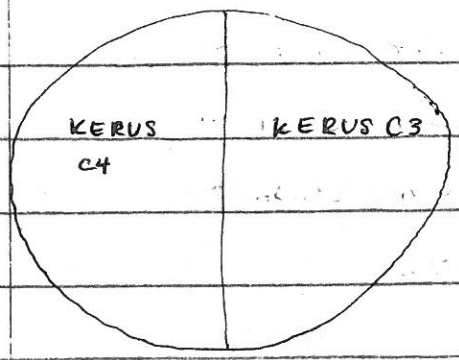
③ 1:100 dilutions of cultures from yesterday, so added 50µL of each culture from yesterday to the new tubes with 5mL LB.

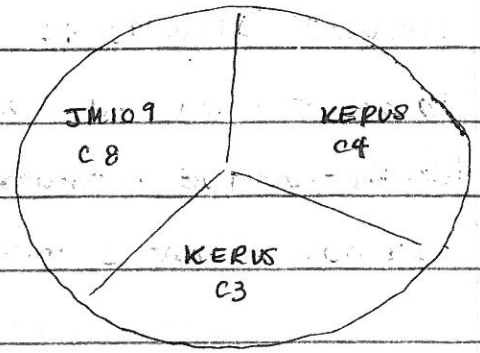
① adding 1ml of C3 and C4 and 10µL of iptg
② place in freezer

① dipped inoculating loop in KerUS C4 and streaked onto 2 LB Amp plates.
② incubated at 37°C

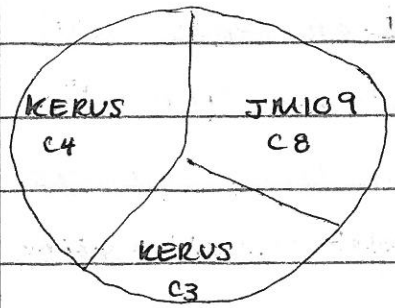
① Plated 10 µl of each culture onto Skim milk AMP plates

Note: the frozen cultures were warmed up in a water bath around 38°C





Frozen H_2O



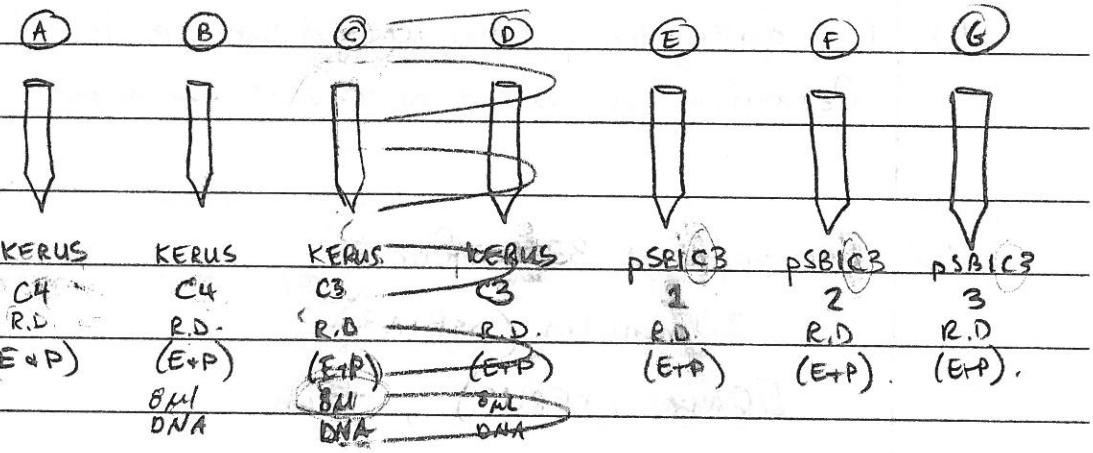
IPTG

② Placed all plates in the incubator at 37°C.

JUNE 2, 2016: J.P,

OBJECTIVE: Restriction digest of frozen KERUS plasmids isolated from Lethbridge May 14th miniprep. Digested DNA to be a) run on a gel electrophoresis to confirm plasmid size and b) be ligated with linearized iGEM plasmid backbones for biobrick assembly + part submission.

(1) Label following small (PCR) tubes:



(2) Thaw (on ice) the plasmid DNA tubes, 10x restriction buffer. Homogenize contents by flicking gently. Collect all liquid in bottom of tube by flicking gently.
KEEP RESTRICTION ENZYMES ON ICE AT ALL TIMES!

(3) Pipette the following into each tube (A-F), in the order listed: (total vol = 20µL)

- 6µL sterile dH_2O
- 10µL plasmid DNA (Ker or pSB1C3) 25ng/µL
- 2µL 10x restriction buffer - NEB2.1
- 1µL Restriction enzyme 1 (EcoR1-HF)
- 1µL Restriction enzyme 2 (PstI)

(4) Mix gently by pipetting up & down a few times. Centrifuge if needed to collect all liquid @ bottom.

(5) Incubate @ 37°C for:

30 minutes (pSB1A3)

50min (KERUS), then:

(6) Incubate @ 80°C in PCR machine for 20min to de-nature restriction enzymes.

(7) Store restriction digests in freezer until ligation or gel electrophoresis.

Web: { PSTI- NEB 3.1
EcoRI-HF Cutsmart } Neb 2.1.

(8) Aliquot 10μL of KERUS C3
10μL of KERUS C4
10μL of pSB1C3
} to freezer for gel.

Friday June 3rd 2016: gel to confirm restriction digest

Objective: Confirm the restriction digest from yesterday (see lab book entry).

① Prepared gel by adding 4mL of 10x TAE to 36mL of dH_2O. (in a graduated cylinder)

② Pour the 1x TAE buffer into an erlenmeyer flask.

③ Weigh out 0.4g agarose and add it to the flask.

④ Place in microwave for 1 minute, removing every 10-15 seconds to swirl.

⑤ Assemble gel cassette by sealing off the gel tray with tape and place comb in proper location.

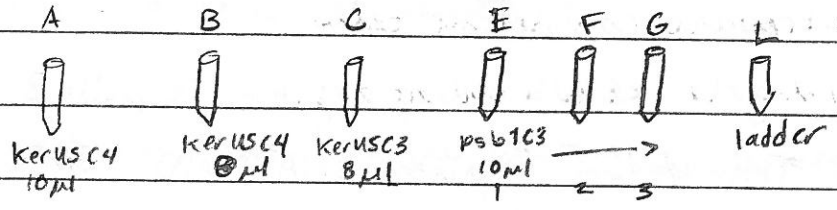
⑥ When the agarose solution is sufficiently clear add 4 µL RedSAFE and mix by swirling (avoid forming air bubbles).

⑦ Pour into gel cassette and wait to solidify

⑧ Prepare DNA sample in a clean microcentrifuge tube by mixing:

- 2 µL Loading Buffer
- 10 µL DNA from restriction digest

Create Samples for:



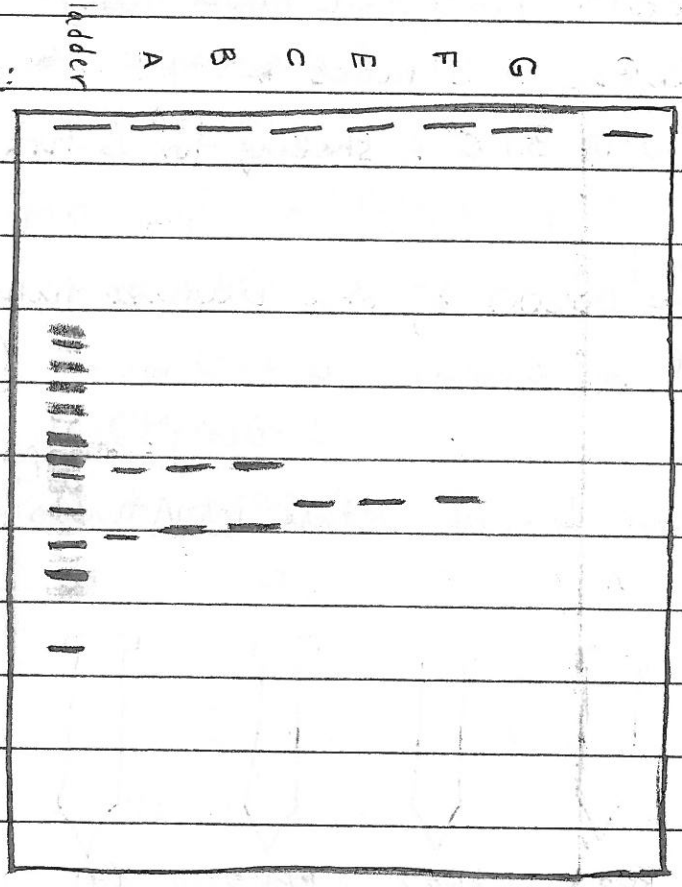
⑨ Fill tank for electrophoresis with 1xTAE buffer.

⑩ Load DNA samples into the wells.

(RECORD which sample went into which well)

⑪ Begin running the gel for 1hr+30 minutes at 100 V.

Results:



FRIDAY, JUNE 10, 2016.

FULL DAY LAB PLAN:

① Prepare competent cells

② Ligate KERUS coding region to pcBIC3 backbon

③ Transform ligation DNA into JM109 E.col

PART ① – PREPARATION OF COMPETENT JM109:

7:45am:

✓ ① Take overnight JM109 culture tube, and transfer 0.1mL (or 100µL) of the culture into 10mL of LB broth (in a 15mL falcon tube).

* Repeat x 3 tubes *

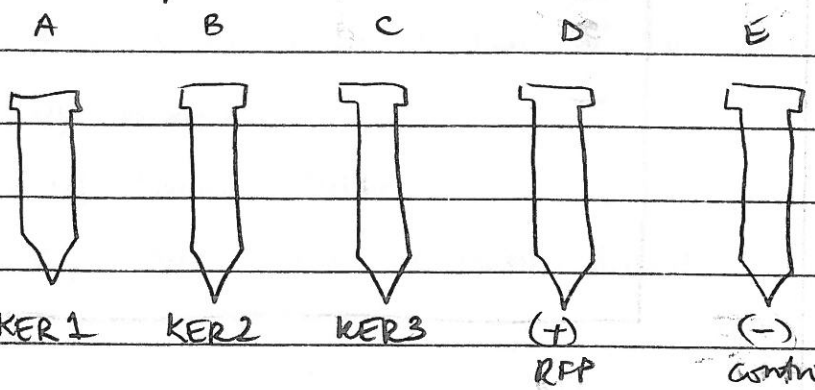
Grow @ 37°C w shaking for 3 hrs.

10:45am:

✓ ② Check OD600 of one culture tube. Optimal level is OD600 = 0.4-0.6.

1.5mL eppendorf

③ Transfer 500µL culture into tubes labelled:



④ Spin down the 6 tubes @ 7000 rpm for 2-3 min. Discard the supernatant.

⑤ Add another 500 µL culture to tubes A-E. Spin down again @ 7000 rpm for 2-3 min. Discard the supernatant. ×2

⑥ Resuspend each pellet in 500 µL Sterile, 50 mM $CaCl_2$. (Pipette up + down to resuspend). Spin down again to get a pellet, and discard supernatant.

⑦ Re-suspend cells in 100 µL of 50 mM $CaCl_2$. Place immediately on ice, wait to Transform!

NOTES or CHANGES:

- OD600 was 0.461
-
-

PART 2 — LIGATION of KERUS to pSB1C3.

9:30am

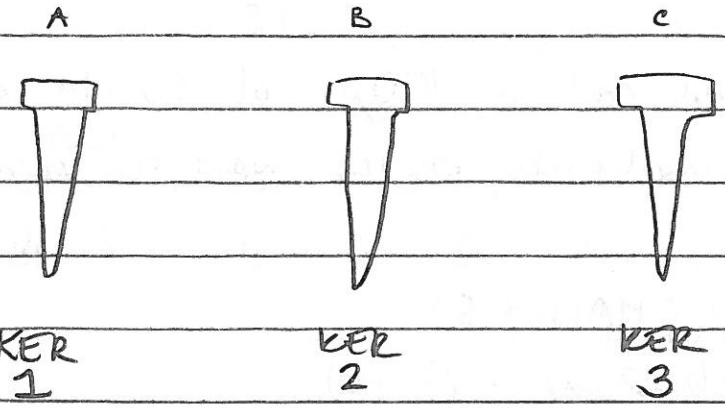
✓ ① Thaw ligase enzyme and ligase buffer ON ICE! Also thaw KERUS and PSB1C3 R. digests from June 3rd. keep on ice!!

✓ ② Rehydrate RFP (+)control from kit plate into PCR tube. Keep on ice

plate: 2016 plate 6 well: 12P

part #: J04450. > Label Tube (1

✓ ③ Label 3 sterile PCR tubes as follows:



✓ ④ Into each tube: (in this order:)

- 3μL sterile dH_2O
- 3μL KERUS DNA
- 2μL pSB1C3 DNA
- 1μL 10× ligation buffer
- 1μL T4 DNA ligase enzyme

✓ ⑤ Mix gently by pipetting up + down. Can spin/flick briefly to collect all liquid

✓ ⑥ Incubate all tubes (NOT tube D!!) at room temp for 1 hour.

✓ ⑦ Incubate all tubes A-C in PCR machine @ 80°C for 20 min.

✓ ⑧ Store tubes A-C on ice until ready to transform

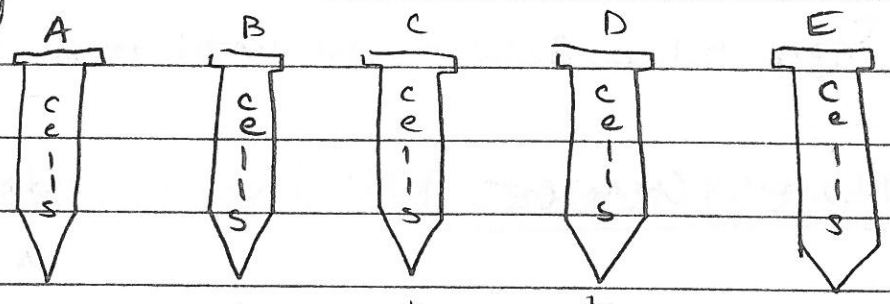
NOTES / CHANGES:

- PCR for 35 min.

PART 3 - TRANSFORMATION:

① Get competent cell aliquots labelled A - E. Thaw slowly on ice until liquid. Turn over gently to mix!

② Add 5μL ligation mix to each tube as follows:



Add	Add	Add	Add	Add
Tube A (KER1) DNA.	Tube B (KER2) DNA	Tube C (KER3) DNA	RFP DNA (rehydrated from kit)	NO DNA.

* Mix tubes gently by flicking.
* Put REST of ligation mix in FREEZER RIGHT AWAY!

✓ ③ Incubate on ice for 30 min. (Tubes A–E).

✓ ④ Incubate at 37°C - water bath - for 5 min.

✓ ⑤ Place immediately back on ice for 5 min.

✓ ⑥ Add 200μL sterile LB broth to each tube (A-E), and mix gently by tapping tube.

⑦ Incubate @ 37°C, w/ gentle rocking, for 2 hours (or until 3:00pm). Meanwhile....

✓ ⑧ Warm up 7 Chlor plates, (room temp) and label them:

plate 1: Ligation A 200μL.
plate 2: Ligation A 20μL.
plate 3: Ligation B 200μL
plate 4: Ligation B 20μL
plate 5: Ligation C 200μL
plate 6: Ligation C 20μL.
plate 7: RFP Control / (–) control. } Divide plate in 1/2, label each half.

⑨ Plate out as indicated, incubate overnight. (wrap in parafilm).

June 23rd 2016 — Miniprep Mrs. P, Nasta, Acacia, Alin

from June 22nd 2016

① Transfecred 2x 700µL into a microcentrifuge tube.

1 — Kerus Biobrick #1 3 — Kerus Biobrick #3

2 — Kerus Biobrick #2 4 — Kerus Biobrick #4

② Centrifuged for ~~2~~ 10 minutes. Poured off supernatant

③ Repeated steps 1-2 w/ 700µL more of culture

new 12

old 34

Friday Sept 23rd

Objective #5: Qualitative Hair/Feather Assay of Biobrick VS. Controls

1. Obtain the following cell culture tubes from objective 4 above:

a. 50µl KERUS overnight + 5mL LB broth + 2.5µl chlor + 50µL IPTG stock

b. Same as tube "a"

c. 50µL KERUS overnight + 5mL LB broth + 2.5µL chlor + 50µL dH2O

d. Same as tube "c"

e. 50µL JM109 overnight + 50mL LB broth + 50µL IPTG stock

f. Same as tube "e"

g. 50 uL JM109 overnight + 50mL LB broth + 50 uL dH2O

h. Same as tube "g"

Set up the following Empty Falcon tubes.

Tube J	Tube K	Tube L	Tube M	Tube N	Tube O
2.5 ml from tube "b" (induced KERUS cells) + Nothing	2.5 ml from tube "b" (induced KERUS cells) + Feathers	2.5 ml from tube "a" (induced KERUS cells) + Hair	2.5 ml from tube "d" (non-induced KERUS) + Nothing	2.5 ml from tube "d" (non-induced KERUS) + Feathers	2.5 mL from tube "c" (non-induced KERUS) + Hair

Tube P	Tube Q	Tube R	Tube S	Tube T	Tube U
2.5 ml from tube "f" (induced JM109 cells) + Nothing	2.5 ml from tube "f" (induced JM109 cells) + Feathers	2.5 ml from tube "e" (induced JM109 cells) + Hair	2.5 ml from tube "h" (non-induced JM109) + Nothing	2.5 mL from tube "h" (non-induced JM109) + Feathers	2.5 ml from tube "g" (non-induced JM109) + Hair

Objective #3

Qualitative assay of Keratinase Activity -

Without cell Lysis: = about 30 minutes

(Plating live cells)

not induced

1. Grow overnight biobrick cultures in LB -chlor broth (5mL) tubes.

2. Also grow overnight cultures of plasmid-less JM109 e.coli cells as a control

3. In the morning, take a Skim milk/chlor plate as shown in diagram below:

4.

① Skim milk + chlor

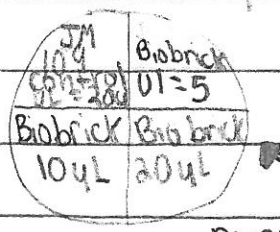
② Skim milk plate only

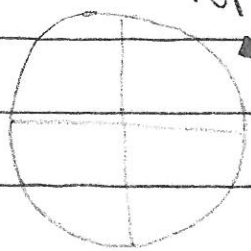
③ Skim milk + chlor + IPTG 10 µL

④ Skim milk only + 10 µL of IPTG stock (Let dry)

Keep the plated cell culture in a small "pool" in the centre of each quadrant and do not jostle the plate or blend. Let grow overnight at room temp or in incubator

40µl 100mM ~~IPTG/plate~~





Objective #4

a qualitative assay of Keratinase Activity - With cell Lysis = 2 hours

Cell Lysis protocal, to obtain supernatant

1. Grow overnight cell cultures for both biobrick parts, and JM109 control cells.

2. The following morning, sub-Culture 8 tubes as follows:

a. 50 µl KERUS overnight + 5mL LB broth + 2.5µl Chlor + 50µl ITPG stock

b. Same as tube "a"

c. 50µl KERUS overnight + 5 mL LB broth + 2.5µl Chlor + 50µl dH2O

d. Same as tube "c"

e. 50µl JM109 overnight + 5mL LB broth + 50µL IPTG Stock

f. Same as tube "e"

g. 50µL JM109 overnight + 5mL LB broth + 50µL dH2O

h. Same as tube "g"

3. Incubate Cultures at 37°C, With shaking, for 3-4 hours

4. Add 1.5 mL of each of the A-H tubes to a 1.5mL eppendorf tube.

KEEP REST OF TUBE CONTENTS FOR OBJECTIVE #5

5. Spin down at 3000 rpm, to obtain a cell pellet. Discard supernatant

6. Add another 1.5mL of the Subculture, and spin down again to obtain a larger pellet. Discard Supernatant

7. Create the following lysis buffer:

a. Stir 0.30g of Tris into 50ml of ddH20.

b. refridgerate for 1 hour

c. add 0.008g of DTT and 0.25mL of lysozyme solution

d. stir gently

8. Add 60µL of the lysis buffer to each of the microcentrifuge tubes

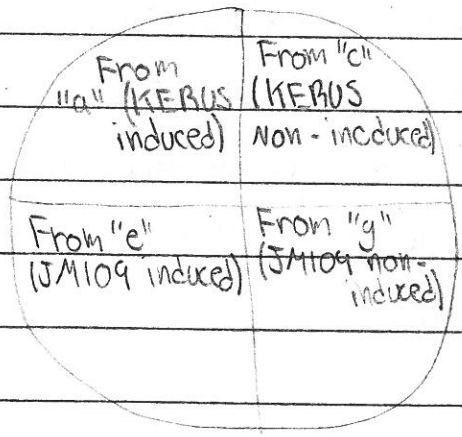
9. Shake tubes in fridge for 30 minutes (or on ice, on shaker table)

10. Place tubes in fridge for 3 minutes, then thaw at 42°C for 3 min (use water bath) Repeat 6 times.

11. Centrifuge tubes again for 2 minutes at 7000 rpm

12. Save Supernatant of each tube. This should contain the KERUS lysed from cells

13. Plate 10µl of each of the supernatants on skim milk chlor plates as follows: (repeat on 2 Plates)



Take care to keep the plated cell culture in a small "pool" in the center of each quadrant, and do not jostle the plate or blend the drops.

Let grow at room temp, or in incubato

Cell Lysis Protocol:

1. Grow overnight colonies of 5mL KerUS in 5μL Chlor and 5mL LB and a control of 5mL DH5α with no Amp and 5 mL LB

2. The next morning, prepare eight falcon tubes Labled A-H:

- A, B; (X2) KerUS 50μL overnight culture + 5mL LB + 5μl of chlor + 50μL IPTG
- C, D; (X2) KerUS 50μL + 5mL LB + 5μL of chlor + 50μL H_2O
- E, F; (X2) 5mL LB + 50μL IPTG
- G, H; (X2) 5mL LB + 50μL H_2O + JM109

3. Incubate cultures for 3.75hours at 37°C

4. Add 1.5mL of each of the A-H tubes to a 1.5 mL Centrifuge tubes

5. Spin tubes for 2 minutes at 7000 RPM
   - Repeat 2X, after pouring off the supernatant, and adding 1.5mL for a bigger pellet

6. Add 60μL of lysis buffer to tubes (see below for lysis buffer protocal)

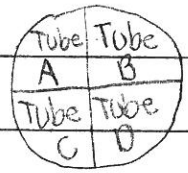
7. Put tubes on ice in incubator

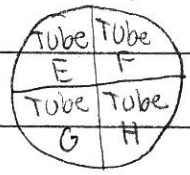
8. Place the tubes on dry ice for 3min then thaw the tubes in the water bath for 3mins at 42°C for 3mins
   - Repeat 6X

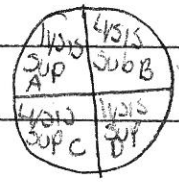
9. centrifuge tubes for 2 mins at 7000 RPM
10. Take supernatants off the pellets and place supernatants into more centrifuge tubes
11. Add 5µL of urea to the pellets for use in an SDS page protin gel

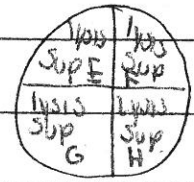
Lysis buffer protocal
1. Add and stir 3.03g of tris into 500mL of demineralization and deionized water
2. Refrigerate for one hour
3. Add 0.077g of DTT and 0.50g of lysozyme to the water of the solution.

LB AMP-Skim milk plating on skim milk plates









→ Most cloudy of pair of tubes for cultures plating 100 µL of broth culture and supernatants

Tubes:

* A+B seeped together

A → KERUS, LB, Chlor, IPTG

B → KERUS, LB, Chlor, IPTG

C → KERUS, LB, Chlor, H_2O

D → KERUS, LB, Chlor, H_2O

* E+F combined

Gmoved close maybe plated too much liquid culture normally Plate 200 µL...

F → JM109, LB, IPTG

F → JM109, LB, IPTG

G → JM109, LB, H_2O

H → JM109, LB, H_2O

Plate 50 µL Lysis Sup (as we learned 100 is too much)